Heleidomermis magnapapula n. sp. (Mermithidae : Nematoda) parasitizing *Culicoides variipennis* (Ceratopogonidae : Diptera) in California

George O. POINAR, Jr. and Bradley A. MULLENS

University of California, Berkeley, CA 94720 and University of California, Riverside, CA 92521, USA.

SUMMARY

Heleidomermis magnapapula n. sp. is described as a parasite of the biting midge, *Culicoides variipennis* (Coquillett) in southern California. The size and shape of the amphidial pouches and spicules and form of the cephalic papillae separate *H. magnapapula* n. sp. from previously described species. Features of the preparasitic juveniles are presented. Host larvae occur in dairy wastewater ponds and the adults are a major vector of bluetongue virus to North American ruminants.

RÉSUMÉ

Heleidomermis magnapapula n. sp. (Mermithidae : Nematoda) parasite de *Culicoides variipennis* (Ceratopogonidae : Diptera) en Californie

Description est donnée de *Heleidomermis magnapapula* n. sp., parasite du Ceratopogonide *Culicoides variipennis* en Californie du Sud. La taille et la forme des poches amphidiennes et des spicules, ainsi que la forme des papilles céphaliques séparent *H. magnapapula* n. sp. des espèces déjà décrites. Les caractéristiques des juvéniles préparasites sont présentées. Les larves hôtes se rencontrent dans les mares contenant les eaux usées des laiteries et les insectes adultes constituent le principal vecteur du virus du " blue tongue " des ruminants en Amérique du Nord.

Mermithid parasitism has been noted on several occasions from biting midges of the genus *Culicoides*, but proper identifications have been rare (see Wirth, 1977). During studies on the biology of *Culicoides variipennis* (Coquillett) in dairy wastewater ponds in southern California, mermithid infections were noted in several populations. After preliminary observations on nematode life history, preserved mermithids and parasitized host larvae were sent to the first author for further life history and systematic studies.

Host larvae of *C. variipennis* were collected from the surface 1-2 cm of mud at the edges of dairy wastewater ponds. Mud was sieved to remove late instar larvae, which were held in tap water in Petri dishes until the mermithids emerged. Some hosts and female mermithids were held individually to determine the number of parasites emerging per host, the duration of oogenesis, and the number of infective juveniles per mermithid female. Larvae of *C. variipennis* and a *Chironomus* sp. were later placed into the Petri dishes to determine the mode of entry. For taxonomic studies, adult nematodes were killed in 60° water, fixed in TAF and processed to glycerin.

The nematodes were determined to be a new species of the genus *Heleidomermis*. In the quantitative portion of the following description, the range of the character is given in parenthesis following the mean value.

Heleidomermis magnapapula n. sp.
(Figs 1-10)

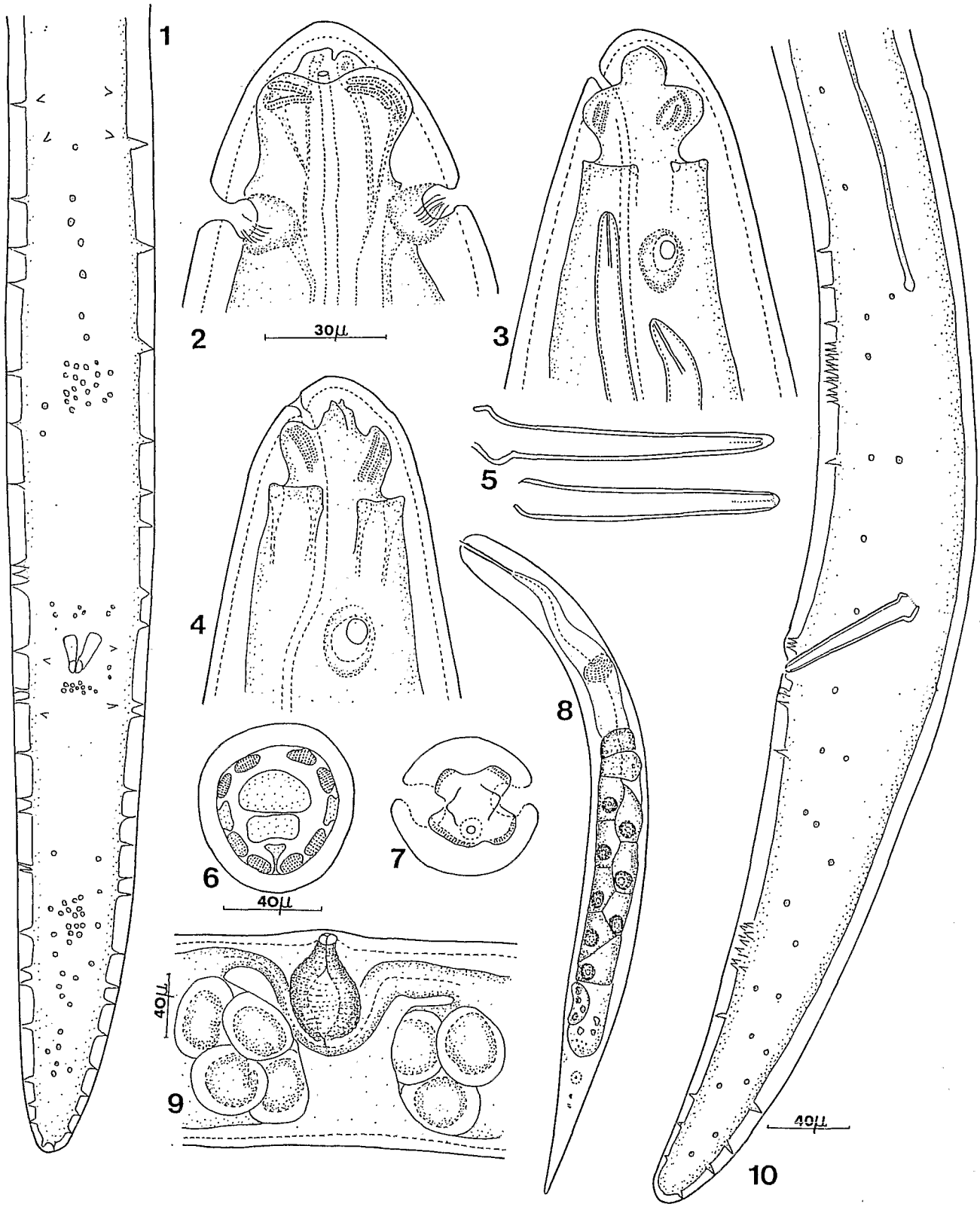
Mermithidae Braun, 1883;
Heleidomermis Rubzov, 1970.

MEASUREMENTS

Females (n = 10) : L = 1.0 cm (0.9-1.4); greatest diameter = 139 µm (107-164); distance from head to nerve ring = 231 µm (206-263); V = 43 (26-58); length and width of vagina = 50 µm (35-65) × 56 µm (42-69); diameter of amphidial opening = 4 µm (3-5); length of amphidial pouch = 9 µm (8-11); egg diameter = 42 µm (32-50) × 52 µm (43-61).

Males (n = 10) : L = 0.5 cm (0.4-0.7); greatest diameter = 82 µm (50-95); distance from head to nerve ring = 224 (206-238); length of spicules = 70 µm (53-83); greatest width of spicule shaft = 8 µm (6-12); length of tail = 234 µm (190-301); body diameter at cloaca = 78 µm (63-89); diameter of amphidial opening = 5 µm (4-7); length of amphidial pouch = 9 µm (8-12).

Holotype (female) : L = 1.1 cm; greatest diameter = 113 µm; distance from head to nerve ring = 263 µm;



Figs 1-10. *Heleidomermis magnapapula* n. sp. 1 : Ventral view of male tail (mag. same as in Fig. 10); 2 : Ventral view of female head; 3 : Lateral view of female head (mag. same as in Fig. 2); 4 : Lateral view of male head (mag. same as in Fig. 2); 5 : Variations in spicule size and shape. The upper spicule is the normal form and the lower one is the most common variation (mag. same as in Fig. 2); 6 : Cross section of male; 7 : Enface view of male (mag. same as in Fig. 2); 8 : Lateral view of infective-stage juvenile (mag. same as in Fig. 2); 9 : Lateral view of vaginal area; 10 : Lateral view of male tail.

V = 41; length and width of vagina = 51 µm × 53 µm; diameter of amphidial opening = 3.2 µm; length of amphidial pouch = 9 µm; egg diameter = 61 µm × 50 µm.

Allotype (male) : L = 0.5 cm; greatest diameter = 76 µm; distance from head to nerve ring = 220 µm; length of spicules = 67 µm; greatest width of spicule shaft = 8 µm; length of tail = 212 µm; body diameter at cloaca = 82 µm; diameter of amphidial opening = 5 µm; length of amphidial pouch = 9 µm.

Infective-stage juveniles (n = 10) : L = 182 µm (164-200); greatest diameter = 13 µm (10-16).

Description

Adults : Small to medium length white nematodes with a smooth cuticle lacking noticeable cross fibers in the adults; adult cuticle 5-8 µm thick at the level of the amphids; head homeocephalic, rounded with two anterior lateral head papillae in one plane and four submedial head papillae arranged in a second plane just posterior to the lateral head papillae. Mouth shifted ventrally, opening near the tips of the lateral head papillae; mouth opening always anterior to the four submedial papillae. Posterior to the four submedial papillae is a hypodermal constriction and just beneath this constriction is a circle of six papillae arranged in dorsal, ventral, lateral ventral and lateral dorsal positions. These papillae range from being highly developed with distinct nerve innervations to being almost rudimentary. They are treated here as neck papillae, rather than being interpreted as part of the head papillae complex. The nerve endings on the submedial papillae are unique and occur in well defined longitudinal bands in contrast to the lateral papillae which are terminated by small papillose nerve endings. The anterior lateral papillae could be considered as mouth papillae and the four submedial papillae as head papillae. The position of the amphids is variable. They never occur anterior to the neck papillae and are sometimes at the same level; usually the openings are posterior to the neck papillae. The amphidial openings are circular and shifted slightly dorsal; amphidial pouch spherical to slightly elliptical; eight hypodermal cords; vulva opening circular; vulva flap absent; vagina barrow-shaped, nearly circular; vagina canal straight, terminating at a junction of opposing branches of the uteri; spicules short, paired, separate, distal tips rounded, proximal portion bent anteriorly; tail long; genital papillae arranged on the ventral, lateral and dorsal (near the tail tip) surfaces; pharyngeal tube continues almost to the cloaca in the male. On some females, a pair of small papillae occurred on the ventral or lateral portions of the tail. Nerve endings running from the hypodermis through the cuticle were common over the body of both sexes.

Postparasitic juveniles : The final molts occur while the nematode is still inside the host. Thus no free-living postparasitic juveniles were found. Shed postparasitic cuticles removed from the host showed no tail appendage.

Infective-stage juveniles : Embryonation, hatching and the first molt occur inside the female nematode. The second stage infective forms emerge from the female through the vulvar opening. The infective stage is short and broad, with a thin stylet, a pair of penetration glands, a stichosome containing eight stichocytes, a small reduced intestine and a genital anlage. A faint anal opening is present.

Type host and locality

Culicoides variipennis (Coquillett) (Diptera : Ceratopogonidae). Dairy wastewater pond on the Aukeman dairy farm, Mira Loma, Riverside Co, California, USA.

Type specimens

Holotype (female) and allotype (male) deposited at the Department of Nematology, University of California, Davis, California. Paratypes deposited at the Laboratoire des Vers, Muséum national d'Histoire naturelle, Paris.

Diagnosis and relationships

There are two previously described species in the highly unusual genus *Heleidomermis*, *H. vivipara* Rubzov, 1970 from Karelia, USSR, and *H. ovipara* Rubzov, 1974 from the Tunkin Valley of the Burjat, USSR. The latter species is oviparous while the former is ovoviviparous. The present species differs from both of the above in both quantitative and qualitative characters. The females of *H. vivipara* are considerably smaller (5.2-6.8 mm) than those of *H. magnapapula* n. sp. (8.6-13.7 mm). The amphidial pouches in *H. magnapapula* n. sp. are nearly spherical measuring only 8-11 µm in both sexes whereas in *H. vivipara* they are 17-20 µm and in *H. ovipara*, 18-20 µm. The difference in the amphidial opening between male and female (4-6 µm *vs* 3-5 µm respectively) is not great in *H. magnapapula* n. sp. but is striking in *H. vivipara* (3 µm in females and 10 µm in males). The egg diameter of *H. magnapapula* n. sp. (32-61 µm) is greater than in *H. vivipara* (30 µm) and *H. ovipara* (30 µm).

Since *H. magnapapula* n. sp. is ovoviviparous, it might be considered most close to *H. vivipara*. However, *H. vivipara* has straight spicules without modified proximal portions, elliptical rather than spherical amphidial pouches, longer infective-stage juveniles and normal cephalic papillae lacking bands of papillary nerve endings.

Discussion

H. magnapapula n. sp. differs from the majority of mermithids in several points. The final molt occurs within the body of the host. The eggs hatch and the first molt occurs within the body of the female. There is an anterior circle of two lateral papillae followed by a lower circle of four morphologically distinct submedial papillae followed by a lower circle of neck papillae.

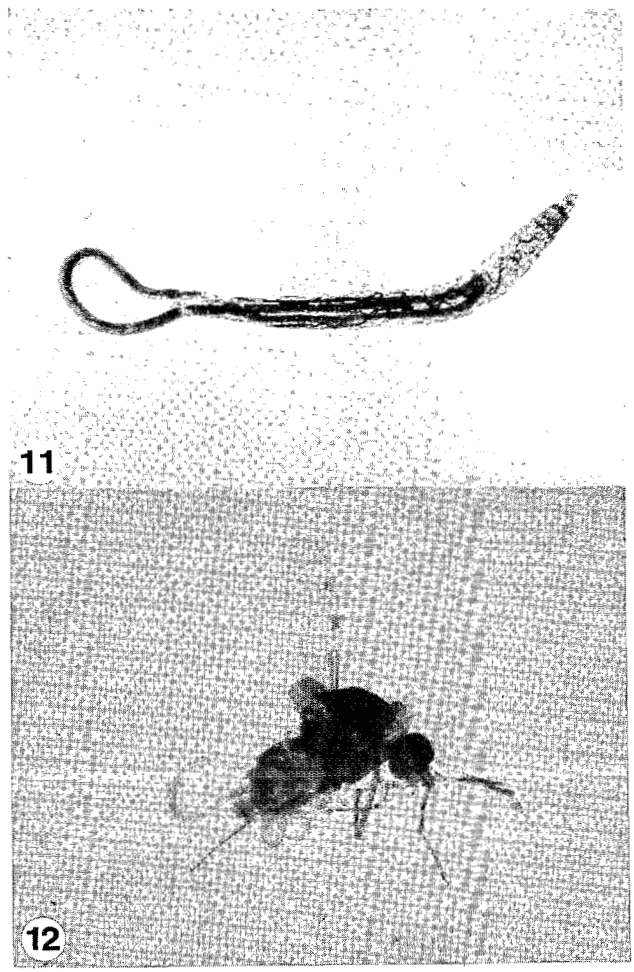
The presence of genital papillae on the lateral and even dorsal tail of the male is equally unusual for mermithids. The infective stages of *H. magnapapula* n. sp. possess only eight stichocytes, a character shared only by some terrestrial mermithids of the genera *Mermis* (Poinar & Hess, 1974) and *Pheromermis* (Kaiser, pers. comm.). It is interesting to note that members of *Mermis* also possess a pair of lateral anterior mouth papillae and a lower circle of four submedial papillae.

The present species shares both spicular and head papillary characters with the *Heleidomermis* reported from *C. variipennis* in similar habitats in New York State (Mullens & Rutz, 1982) and the New York population is thus also considered to be *H. magnapapula* n. sp. The occurrence of *Heleidomermis* in populations of *C. variipennis* appears to be widespread in the United States since aside from California and New York, this host-parasite relationship has been found in horse pens in Alabama (Hribar, pers. comm.) and highly saline habitats in southwestern Virginia (Zimmerman, pers. comm.).

Biological notes

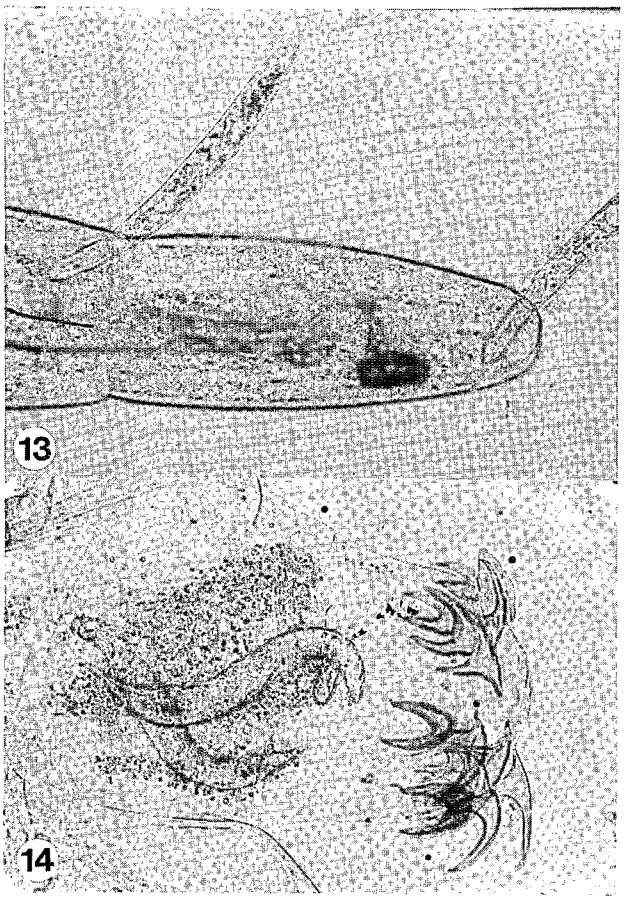
Infected larvae of *C. variipennis* were collected from dairy wastewater ponds at four farms in southern California. Two of the four ponds were permanent, while the other two held water for six to nine months prior to sampling. All of the ponds had some level of manure loading, which favored high host populations. Parasitism at one pond, which held water for at least a year prior to sampling, was approximately 10 % in late September. The rate of parasitism can vary seasonally, however, as discussed by Mullens and Rutz (1982).

It is likely that the high salt and ammonium content provides a harsh habitat, yet one that is tolerated, for the nematode. This is perhaps why certain modifications of the life cycle have occurred in *H. magnapapula* n. sp. Maturation to the adult stage inside the insect restricts the time of contact between the mature mermithids and the external environment. The remainder of the cycle is also accelerated since mating occurs immediately after host emergence and the infective stages hatch 4-6 days later (at 20°; 8-9 days at 15°) and can penetrate a host as soon as they leave the female nematode. Embryogenesis and hatching within the mother nematode can be regarded as a further adaptation to limit contact of the infective juveniles with the manure-laden water. In tap water at 20°, the infective juveniles survived 4-6 days.



Figs 11-12. 11 : Adult female of *Heleidomermis magnapapula* n. sp. emerging from a fourth instar larva *Culicoides variipennis;* 12 : Adult *Culicoides variipennis* containing a developing juvenile of *Heleidomermis magnapapula* n. sp.

When placed together with second and third stage larvae of *C. variipennis*, the infective stages rapidly penetrated through the larval cuticle and entered the hemocoel (Fig. 13). Such aggressiveness was also noted to occur when second and third stage *Chironomus* larvae were also placed in the infection chamber. Penetration occurred normally but the juveniles were killed within two hours by a melanization reaction (Fig. 14). The duration of parasitic development was not noted but based on host life history, is judged to be about 14 days. Most of the mature nematodes emerge from fourth instar host larvae (Fig. 11) but emergence was also noted to occur from third instar larvae and pupae. Several adult *C. variipennis* were observed to harbor mermithids as well



Figs 13-14. 13 : Two juveniles of *Heleidomermis magnapapula* n. sp. penetrating the cuticle of a second instar *Culicoides variipennis* larva; 14 : Two juveniles of *Heleidomermis magnapapula* n. sp. that were melanized after entering the hemocoel of a second stage *Chironomus* sp. larva.

Accepté pour publication le 27 janvier 1987.

(Fig. 12) and this is probably the main vehicle of dispersal for *H. magnapapula.* Though prior studies in New York did not yield more than two parasites/host (Mullens & Rutz, 1982) as many as six nematodes emerged from a single host larva in the present studies. In cases where hosts harbor both male and female mermithids, it is possible that the pair could mate while still within the insect. Isolated female mermithids did not produce developing eggs whereas thirteen mated females produced an average of 1 709 (1 099-2 553) infective juveniles each.

The host, *C. variipennis,* is a major vector of bluetongue virus to North American ruminants. It is unclear at present whether parasite introductions or cultural practices might increase the level of *Culicoides* parasitism in wastewater ponds. The hosts can be produced in large numbers in laboratory colonies, so the possibility of mass-culturing the parasite exists. However, this nematode lacks a life stage suitable for long-term storage or easy field transport. Further studies may elucidate cultural factors (e. g. adjusting the level of manure loading) that would favor parasite activity.

References

Mullens, B. A. & Rutz, D. A. (1982). Mermithid parasitism in *Culicoides variipennis* (Diptera : Ceratopogonidae) in New York State. *Mosquito News,* 42 : 231-235.

Poinar, Jr., G. O. & Hess, R. (1974). Structure of the preparasitic juveniles of *Filipjevimermis leipsandra* and some other Mermithidae (Nematodea). *Nematologica,* 20 : 163-173.

Rubzov, I. A. (1970). [A new species and genus of mermithids from biting midges.] In : *Novye i maloizvestnye vidy foemy Sibiri.* SO izd. Nauka, Novosibirsk, 3 : 94-101.

Rubzov, I. A. (1974). [New species of mermithids from midges.] *Parasitologiya,* 8 : 212-219.

Wirth, W. W. (1977). A review of the pathogens and parasites of the biting midges (Diptera : Ceratopogonidae). *J. Wash. Acad. Sci.,* 67 : 60-75.